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(54) Abstract Title

**Presentation of Polyunsaturated Fatty Acids**

(57) A non-aqueous formulation of a fatty acid anhydride conforming to the general formula R<sup>1</sup>-O-R<sup>2</sup>, where R<sup>1</sup> and R<sup>2</sup> are polyunsaturated (C<sub>16</sub>-C<sub>26</sub>) fatty acid acyl groups, is particularly efficacious for the presentation of the corresponding acids in therapy or as food additives. Ordinary or mixed acid anhydrides are chosen from a group of acids including octa(poly)enoic, eicosa(poly)enoic and docosa(poly)enoic acids. Preferred acids are those with two to six cis double bonds.

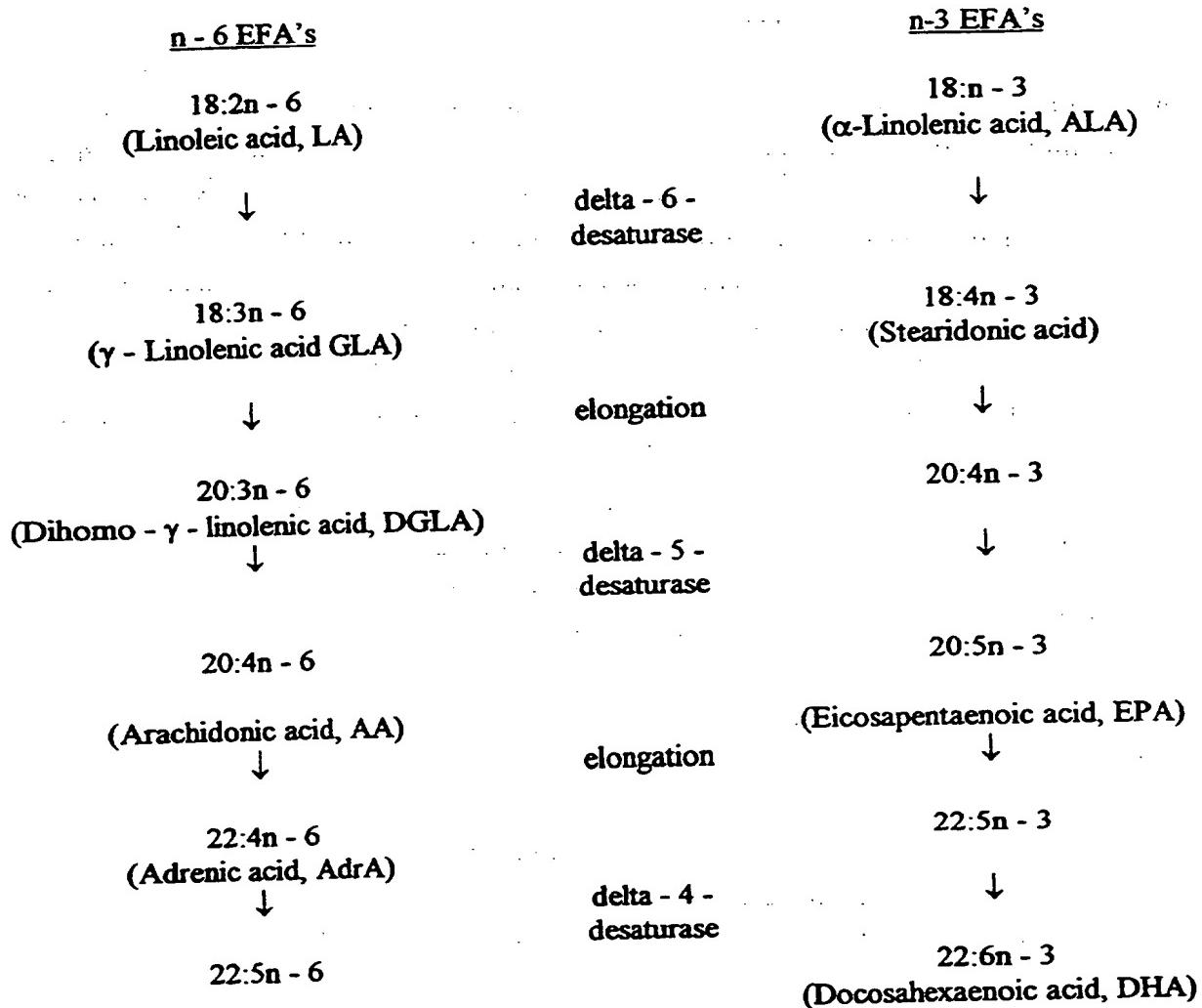
GB 2 323 031 A

**PRESENTATION OF FATTY ACIDS**

The invention relates to presentation of fatty acids.

This invention particularly relates to the presentation of bioactive polyunsaturated fatty acids, including fatty acids which contain 16 - 26 carbon atoms and which have 2 to 6 double bonds in either the cis or trans configuration. It more particularly relates to fatty acids of 18 to 22 carbon atoms with 2 to 6 double bonds all in the cis configuration. Such fatty acids include linoleic acid (18:2 n-6, LA), gamma-linolenic acid (18:3 n-6, GLA), dihomogamma-linolenic acid (20:3 n-6, DGLA), arachidonic acid (20:4 n-6, AA), adrenic acid (22:4 n-6, AdrA), alpha-linolenic acid (18:3 n-3, ALA) stearidonic acid (18:4 n-3, SA), eicosapentaenoic acid (20:5 n-3, EPA), docosapentaenoic acid (22:5 n-3, DPA) and docosahexaenoic acid (22:6 n-3, DHA). These n-6 and n-3 series essential fatty acids are related as follows:-

**TABLE 1**



The acids, which in nature are of the all - cis configuration, are systematically named as derivatives of the corresponding octadecanoic, eicosanoic or docosanoic acids, e.g. z,z-octadeca - 9,12 - dienoic acid or z,z,z,z,z,z - docosa- 4,7,10,13,16,19 - hexaenoic acid, but numerical designations based on the number of carbon atoms, the number of centres of unsaturation and the number of carbon atoms from the end of the chain to where the unsaturation begins, such as, correspondingly, 18:2 n-6 or 22:6 n-3, are convenient. Initials, e.g. EPA, and shortened forms of the name e.g. eicosapentaenoic acid, are used as trivial names in some instances.

Other possible acids include columbinic acid or parinaric acid or conjugated linoleic acid or conjugated octadecatrienoic acid or alpha-eleostearic acid.

These fatty acids have biological and therapeutic effects which in themselves are well documented in the literature and in many previous patent applications by the present applicants. When developing such fatty acids or their derivatives as agents for pharmaceutical use or for nutritional use either as nutritional supplements or as substances to be added to foodstuffs there are several important considerations:

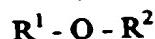
1. The fatty acid or derivative should be well tolerated when given orally or parenterally. This requirement rules out the free fatty acids themselves for most purposes since in other than carefully controlled quantities they cause gastrointestinal irritation when given orally or blood cell dissolution when given intravenously.
2. The fatty acid derivatives should be well absorbed and metabolised along normal metabolic pathways.
3. In some situations two or three fatty acids with similar or different biological actions need to be incorporated into a single molecule. This is particularly important in pharmaceutical situations where it may be important simultaneously to provide two or three bioactives within a single chemical entity.

Recent filings from the applicants have described in detail various fatty acid derivatives that may be used with these ends in mind, and anhydrides are briefly mentioned in PCT/GB96/01053, published as WO96/34846. In the course of testing a range of fatty acid derivatives we have made the surprising observation that anhydrides are extremely well tolerated, absorbed and metabolised. Consistently the anhydrides perform better than other fatty acid derivatives in raising blood levels of the constituent fatty acids. These anhydrides are thus preferred derivatives for pharmaceutical use in particular, although they also have value as nutritional supplements in situations such as encapsulated products where water is excluded. On addition of water to these compounds they dissociate into free fatty acids.

This may explain why they are so effective when taken by mouth since no digestive processes are required to allow them to be absorbed into the body.

### The Invention

The invention therefore lies in compounds of the following acid anhydride structure, when for use in therapy in non-aqueous formulations, particularly for securing rapid uptake in the constituent fatty acids of the blood plasma and red cell lipids:-



where  $\text{R}^1$  is an acyl group derived from a bioactive unsaturated fatty acid particularly a  $\text{C}_{16-26}$  preferably  $\text{C}_{18-22}$  fatty acid with two to six *cis* or *trans* double bonds, and  $\text{R}^2$  is an acyl group as  $\text{R}^1$ , the same or different.

The invention further lies in the compounds for use as nutritional supplements or food additives in non-aqueous formulations.

Particular fatty acids are as set out earlier herein and the acids may be provided in daily doses of 1 mg to 200 g, preferably 50 mg to 20 g and very preferably 300 mg to 5 g, except in cancers where the higher dose ranges may be required. The preferred route of administration is oral, in gelatin or other capsules but other routes and formulations may be applied by those skilled in the art, provided the formulation is non-aqueous in the sense of protecting the anhydrides from hydrolysis by access of water before administration.

### Synthetic Routes

The individual fatty acids may be purified from natural animal, vegetable or microbial sources or may be chemically synthesised, all by methods known to those skilled in the art or to be developed hereafter.

Synthesis of the claimed compounds requires the formation of an acid anhydride. Such chemistry may be achieved by any reasonable method of anhydride synthesis and especially:

- (a) by reaction of a fatty acid with a dehydrating agent, e.g. 1,3-dicyclohexylcarbodiimide or acetic anhydride, with or without an inert solvent, e.g. methylene chloride, at a temperature between -20°C and 200°C
- (b) by reaction of an alkali metal or alkaline earth metal or silver salt of a fatty acid with a fatty acid chloride or other suitable activated fatty acid derivative with or without an inert solvent, e.g. methylene chloride, at a temperature between -20°C and 50°C.
- (c) by reaction of a fatty acid and a fatty acid chloride with or without the presence of an organic tertiary base, e.g. pyridine and with or without an inert solvent, e.g. hexane, at a temperature between -20°C and 100°C.
- (d) by reaction of a fatty acid chloride with an appropriate quantity of water in the presence of an organic tertiary base, e.g. pyridine, with or without a suitable inert solvent, e.g. hexane, at a temperature between -20°C and 50°C.

#### **Preparative examples**

##### **Example 1**

*z,z,z-octadeca-6,9,12-trienoic anhydride*

*(GLA anhydride)*

A mixture of *z,z,z-octadeca-6,9,12-trienoic acid* and *1,3-dicyclohexylcarbodiimide* (0.55 equiv.) in hexane was stirred overnight under nitrogen at room temperature. The reaction mixture was filtered to remove precipitated *1,3-dicyclohexylurea*. The filtrate was stored overnight at -20°C to precipitate any residual *1,3-dicyclohexylurea* which was again removed by filtration. Concentration under reduced pressure yielded *z,z,z-octadeca-6,9, 12-trienoic anhydride* as a pale yellow, free flowing oil. Infra red analysis indicated two peaks at 1820cm<sup>-1</sup> and 1750 cm<sup>-1</sup>, consistent with an anhydride grouping.

**Example 2**

*z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic anhydride*  
*(EPA anhydride)*

Reaction of *z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic acid* and *1,3-dicyclohexylcarbodiimide* under the conditions given in Example 1 yielded *z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic anhydride* as a pale yellow, free flowing oil. Infra red analysis indicated two peaks at  $1820\text{ cm}^{-1}$  and  $1750\text{ cm}^{-1}$ , consistent with an anhydride grouping.

**Example 3**

*z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic, z,z,z-octadeca-6,9,12-trienoic mixed anhydride*  
*(GLA-EPA mixed anhydride)*

*N,N-diisopropylethylamine* was added dropwise to a mixture of *z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyl chloride* and *z,z,z-octadeca-6,9,12-trienoic acid* in hexane at room temperature. After stirring overnight at room temperature under nitrogen, the mixture was filtered to remove *N,N-diisopropylethylammonium chloride*. Concentration under reduced pressure yielded *z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic, z,z,z-octadeca-6,9,12-trienoic mixed anhydride* as a yellow, free flowing oil. Infra red analysis indicated two peaks at  $1820\text{ cm}^{-1}$  and  $1750\text{ cm}^{-1}$ , consistent with an anhydride grouping.

**Example 4**

*z,z,z,z,z,z-docosa-4,7,10,13,16,19-hexaenoic, z,z,z-octadeca-6,9,12-trienoic mixed anhydride*  
*(GLA-DHA mixed anhydride)*

Reaction of *z,z,z,z,z,z-docosa-4,7,10,13,16,19-hexaenoyl chloride* and *z,z,z-octadeca-6,9,12-trienoic acid* under the conditions given in Example 3 yielded *z,z,z,z,z,z-docosa-4,7,10,13,16,19-hexaenoic, z,z,z-octadeca-6,9,12-trienoic mixed anhydride* as a yellow, free flowing oil. Infra red analysis indicated two peaks at  $1820\text{ cm}^{-1}$  and  $1750\text{ cm}^{-1}$ , consistent with an anhydride grouping.

## Formulations

Among suitable formulations are soft or hard gelatin capsules containing 50 to 1000 mg of anhydride (Examples 1 to 4), taken for example 2 capsules 3 times a day.

## Experimental

The biological efficacy of the anhydrides was illustrated as follows. Rats were put on a fat free diet to which was added 0.1% of GLA in six different forms, triglyceride-GLA (TriGLA); 1,3 propane diol GLA diester (DiGLA); 1,3 propane diol GLA monoester (GLA monester); ascorbyl-GLA; niacin-GLA, and the GLA-GLA anhydride. After two weeks blood samples were taken and the GLA derivatives ranked according to their ability to raise the levels of GLA itself and of its main metabolite, DGLA, in the phospholipids and cholesterol esters of plasma and the phospholipids of the red cell membrane. For all three GLA fractions, the GLA-GLA anhydride was the most effective compound in raising GLA levels. It was also the most effective compound in raising the levels of DGLA in all three fractions, showing that the GLA in the anhydride is not only the derivative which is most effectively absorbed but also the one which is most effectively metabolised to DGLA. Similar but less extensive results were obtained with the EPA-EPA anhydride and the mixed GLA-EPA and GLA-DHA anhydrides. The anhydrides are therefore an unexpectedly favourable way to administer one or two bioactive unsaturated fatty acids.

**Table 1.** Ranking \*\* of the efficiency of various GLA derivatives in raising blood levels of GLA and DGLA when provided at 0.1% of rat diet. PPL plasma phospholipid; PCE plasma cholesterol ester; RPL red cell phospholipids.

	GLA PPL	GLA PCE	GLA RPL	DGLA PPL	DGLA PCE	DGLA RPL
<b>GLA-GLA anhydride</b>	1	1	1	1	1	1
<b>TriGLA</b>	6	5	6	6	3	6
<b>DiGLA</b>	3	3	4	5	5	5
<b>GLA monoester</b>	2	2	5	3	4	4
<b>Ascorbyl-GLA</b>	5	6	2	2	2	3
<b>Niacin-GLA</b>	4	3	2	3	6	2

\*\* Arbitrary 1 - 6 scale (1 most effective, 6 least effective)

## CLAIMS

1. Compounds of the following acid anhydride structure, when for use in therapy in non-aqueous formulations, particularly for securing rapid uptake in the constituent fatty acids of the blood plasma and red cell lipids:-



where  $\text{R}^1$  is an acyl group derived from a bioactive unsaturated fatty acid particularly a C<sub>16-26</sub> preferably C<sub>18-22</sub> fatty acid with two to six *cis* or *trans* double bonds, and  $\text{R}^2$  is an acyl group as  $\text{R}^1$ , the same or different.

2. A compound according to claim 1, wherein the fatty acids are selected from the n-6 and n-3 series essential fatty acids and, columbinic acid, parinaric acid, conjugated linoleic acid, conjugated octadecatrienoic acid and alpha-eleostearic acid.

3. A compound according to claim 2, wherein the fatty acids are selected from linoleic acid, gamma-linolenic acid, dihomogamma-linolenic acid, arachidonic acid, adrenic acid, alpha-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosapentaenoic acid n-3 and docosahexaenoic acid.

4. Compounds as in claims 1 - 3 for use as nutritional supplements or food additives in non-aqueous formulations.



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Patent  
Office

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Application No: GB 9805123.8  
Claims searched: 1-4

Examiner: Simon M. Fortt  
Date of search: 18 May 1998

**Patents Act 1977**  
**Search Report under Section 17**

**Databases searched:**

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.P): A5B (BHA, BJA)

Int Cl (Ed.6): A61K 31/185, 31/20

Other: On-line: CAS-ONLINE, WPI.

**Documents considered to be relevant:**

Category	Identity of document and relevant passage	Relevant to claims
A	WO 96/34846 A1 (SCOTIA HOLDINGS PLC) whole document	

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|-------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| X Document indicating lack of novelty or inventive step                                                     | A Document indicating technological background and/or state of the art.                                            |
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